

THE OSSIFICATION OF THE BASISPHENOID AND PRESPHENOID BONES IN
MELOPSITTACUS.

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(Plates viii-x; two Text-figures.)

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In two recent communications the writer (Kesteven, 1940, 1942) has set out evidence in favour of the view that the so-called parasphenoid of the Sauria is in reality only part of the cartilage-replacing basisphenoid bone.

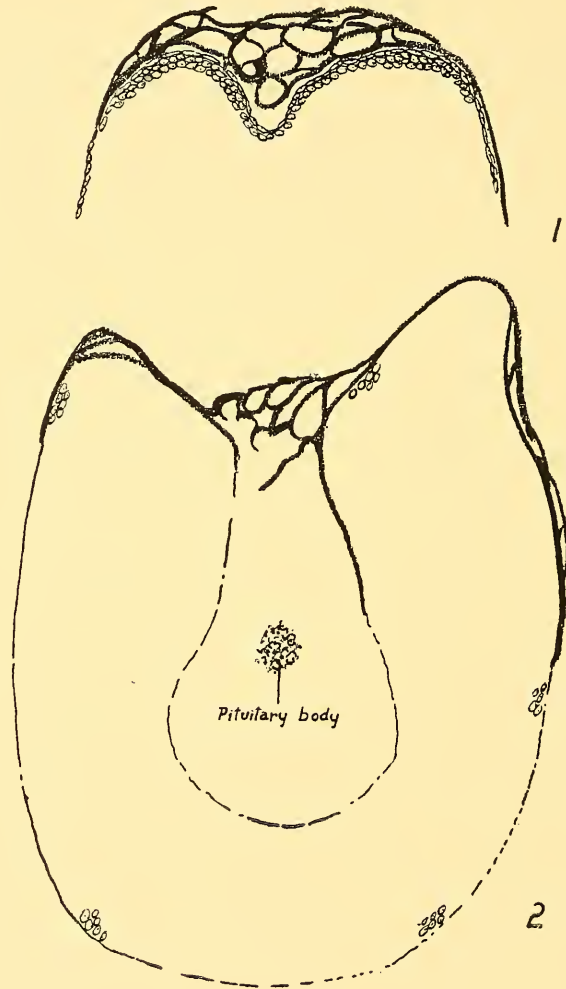
After the latter of the two works had gone to the press, Mr. M. Brennan presented me with three more stages in the development of *Melopsittacus*; these were the seven-, eight- and nine-day chicks. The eight-day chick presents the whole history of the development of the presphenoid, which, in the past, has been regarded as the vomer, and the basisphenoid bones.* This presentation of the whole history is, of course, due to the fact that the bone commences to develop beneath the pituitary fossa first of all, and then the process of ossification extends forward and laterally. One, therefore, finds right forward, at the anterior end of the septum intorbitale, that the ventral edge of the cartilage and its perichondrium show no trace of any of the changes which precede ossification. As the sections are studied, passing backwards, the earliest of those changes present themselves, and then the succeeding changes may be studied in the following sections. In this eight-day chick of the Love Parakeet the changes in question present, in almost diagrammatic clarity, the process of ossification which the writer described in the latter of the two papers referred to.

In view of the importance of the question, affecting, as it does, in a fundamental manner, the interpretation of the bones on the base of the skull, not only of the Sauria, but also of the Theria, it has been thought well worth while to present this further evidence in support of the new interpretation of these bony lamellae on the base of the embryonic saurian skulls.

Prior to the inception of any changes leading to ossification, the ventral edge of the septum intorbitale is essentially similar to the rest of the cartilage. The deeper portions are typical, rounded, embryonic cartilage cells; towards the surface the cells are oval in outline, with the long axis parallel with the surface; the outermost two or three layers are flattened. Outside these, and directly in contact with them, lies the perichondrium of two or three layers of flattened cells. With the inception of ossific changes the perichondrial cells against the ventrum and a little way up on each side of the cartilage enlarge and become oval to spherical. A little later in the process, or further back along the sections, a thickening of the perichondrium below the cartilage becomes manifest. This thickening is, with little doubt, due to proliferation of the perichondrial cells (Pl. viii, fig. 1). The series of sections was stained with iron haematoxylin and counterstained with van Gisson's picro-fuchsin. The cartilage has taken only the blue of the haematoxylin, but with a faint brown tinge in the nuclei of the cartilage cells. Connective tissue nuclei are stained a very dark blue, appearing almost black, and connective tissue fibrils are also deeply stained in the same way. The cytoplasm of the perichondrial cells is stained by the fuchsin, and their nuclei by a combination of the picric acid and haematoxylin, and these are brown in colour. There is, therefore, quite a marked contrast between the cartilage and its perichondrium. When the perichondrial cells increase in size this contrast becomes even more marked, because the affinity of their cytoplasm for the fuchsin gives them a reddish-violet colour.

* The sections described below are in my own private collection, and are labelled "Budgerygar Ser. No. 2".

Coincident with the early activity of the perichondrium, one observes the development of a lacuna, probably lymph filled, below the ventral edge of the cartilage. The outer wall of this lacuna appears to be formed by flattened connective tissue cells, but I have been unable to make quite sure that the cells in question are not the outermost layer of the perichondrium. At first this lacuna lies ventrally to the thickness of the perichondrium, but rapidly extends upward so that only a single layer of cells intervenes between it and the cartilage (Pl. viii, fig. 2). Almost at once further proliferation of the perichondrium results in the filling of the lacuna, and a "cushion" of perichondrial cells is developed along the ventral edge of the cartilage (Pl. viii, figs. 3 and 4).



Text-figs. 1 and 2.

At first the whole of the cells in this cushion are similar to one another and are of the reddish-violet colour; soon, however, those centrally situated lose their affinity for fuchsin and take instead the yellow stain of the picric acid, and these cells, though larger, are not so closely packed as the others.

At the same time the cells at the ventral periphery of the cartilage show the first changes of degeneration which precede replacement by bone, patches of them losing their regular outline.

These changes are soon followed by the appearance of the first bony spicules; these stain an intense fuchsin red and are found in the centre of the yellow-stained osteoblasts (Pl. viii, fig. 5).

At first the osteoblasts in contact with the bony spicules present no obvious difference from the rest, but very soon the deposition of bone all round them imparts to them very definite outlines. A few of these definitely-enclosed cells are to be found almost from the first (Pl. viii, fig. 5; Pl. ix, fig. 6).

The figures in Plates viii and ix have all been drawn to the same scale, and from these it is clear that the "cushion" steadily increases in depth as well as in width as the cartilage thickens.

There is continuous proliferation of the osteoblastic cells to produce this increased size of the cushion, but in addition, as ossification proceeds, the osteoblastic tissue is invaded by vascularized connective tissue, the first appearance of the Haversian canals (Pl. ix, fig. 7). With increasing formation of bone, more and more of the osteoblasts come to be completely enclosed, and the little clusters of these cells have a striking resemblance to cartilage.

Meanwhile, the ventral portion of the cartilage has been undergoing further degeneration, the large majority of the cells are vacuolated and their cytoplasm and nuclei contracted and distorted.

In Plate x, fig. 8, which illustrates the ventral portion of the interorbital septum, towards its posterior end where the two trabeculae are fused, the anterior end of the basisphenoid bone is illustrated; this section is cut just a little forward of the pituitary fossa. Here the process of ossification has progressed as far as it has gone in the eight-day chick. The ventral portion of the cartilage has undergone extensive degeneration. All the cells are now shrunken and distorted and lie in irregular, large rounded spaces in the partially-dissolved cartilaginous matrix. There are now present amongst the osteoblasts close to the cartilage a few large oval cells, with large nuclei, which have been regarded as chondroclasts.

Another feature of interest in this section is that one sees here the commencement of that loss of definition of the outer edge of the osteoblastic tissue and the invasion of the mesoblastic connective tissue by the osteoblasts, which was described in the previous papers.

Text-figures 1 and 2 are purely diagrammatic, and are intended to show the extension of the ossification in relation to the inner and outer surfaces of the walls of the pituitary fossa. There can be no doubt whatever that this is not a parasphenoid, but is quite definitely a basisphenoid ossification.

No suture has been found between the presphenoid and basisphenoid ossification, nor was one expected. On the other hand, it has to be admitted that even if such a suture were present it would be exceedingly difficult to recognize in transverse sections.

References.

- KESTEVEN, H. LEIGHTON, 1940.—The Osteogenesis of the Base of the Saurian Cranium and a Search for the Parasphenoid Bone. *PROC. LINN. SOC. N.S.W.*, 65, 447-467.
 ———, 1942.—The Ossification of the Avian Chondrocranium, with Special Reference to that of the Emu. *Ibid.*, 67, 213-237.